Flight System

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HAZLETON LABORATORIES

INCORPORATED • FALLS CHURCH, VIRGINIA

MONTHLY PROGRESS REPORT NO. 3

A STUDY TOWARD DEVELOPMENT OF AN AUTOMATED MICROBIAL METABOLISM LABORATORY

Contract No. NASW-1507

Submitted to

National Aeronautics and Space Administration
Washington, D. C.

January 1, 1967

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A. BIOCHEMISTRY

1. Phosphate Uptake by Escherichia Coli

An E. coli cell suspension was inoculated into 40 ml. of low-phosphate (1 mg. PO_4-P per liter) M9 medium containing 0.2% glucose. After 21 hours of incubation, a portion of the culture was transferred to fresh, low-phosphate medium to initiate a second culture. Three hours later, the first culture became 24 hours old (stationary phase) and its subculture became three hours old (log phase). The cell density in each culture was roughly determined from an O. D. curve. One ml. (approximately 3.5×10^8 cells) of 24-hour culture and 1.0 ml. (approximately 1×10^8 cells) of three-hour culture were centrifuged. The 24-hour cells were washed three times with sterile saline solution, resuspended in saline and serially diluted to prepare 0.1 ml. aliquots containing approximately 1.6×10^6, 1.6×10^5 and 1.6×10^4 cells. These aliquots were inoculated into 40 ml. portions of low-phosphate M9 medium to give 4×10^4, 4×10^3 and 4×10^2 cells per ml., respectively. One-tenth ml. aliquots of the three-hour inoculum containing 1×10^6, 1×10^5 and 1×10^4 were similarly inoculated into 40 ml. low-phosphate M9 medium to yield 2×10^4, 2×10^3 and 2×10^2 cells per ml., respectively. All cultures were prepared in duplicate. Duplicate controls containing 4×10^4 cells per ml., in the case of the 24-hour culture, and 2×10^4 cells, in the case of the three-hour culture, were treated with 2,4-dinitrophenol (DNP) at 0.66 mM. All the cultures were aerated at a rate of 92 ml. per minute while incubating at 37° C. Actual cell counts were determined by Tryptic Soy Agar (TSA) plates made from aliquots of the inocula.

Aliquots from each incubating culture were taken at 0 time, five hours and 24 hours. They were filtered by membrane filter and the filtrate was assayed for dissolved orthophosphate. TSA counts were made at 0 time and at 24 hours. The results are shown in Figure No. 1. In all cases, except the controls, phosphate uptake was demonstrated in five hours, the earliest sampling after time 0.

Phosphate uptake was always higher in the physiologically younger cultures. This is consistent with results given in Progress Report No. 2. In both the three-hour and 24-hour inocula cultures, the greatest phosphate uptake consistently occurred in cultures of smallest inoculum. Since both types of cultures attained cell numbers of 10^8 per ml., more cell mass had been produced from the lower inocula which, hence, required more phosphate. The lesser phosphate uptake shown by the DNP-treated cultures was the result of uncoupling of oxidative phosphorylation, demonstrating that the uptake was biological in nature. The small amounts of phosphate taken up by these cultures can probably be ascribed to substrate phosphorylation by the inhibited cells.

To compare phosphate uptake between aerated and non-aerated cultures of E. coli, a similar experimental design as described above was conducted. However, this time, all the cultures were incubated in a static condition at 37° C. Bard-Parker (BP), a germicidal disinfectant, was used in the controls (3.0 ml. BP + 37.0 ml. M9 medium). Under these conditions, clearly demonstrable phosphate uptake occurred between the five- and 24-hour samplings. Details are given in Figure No. 2.

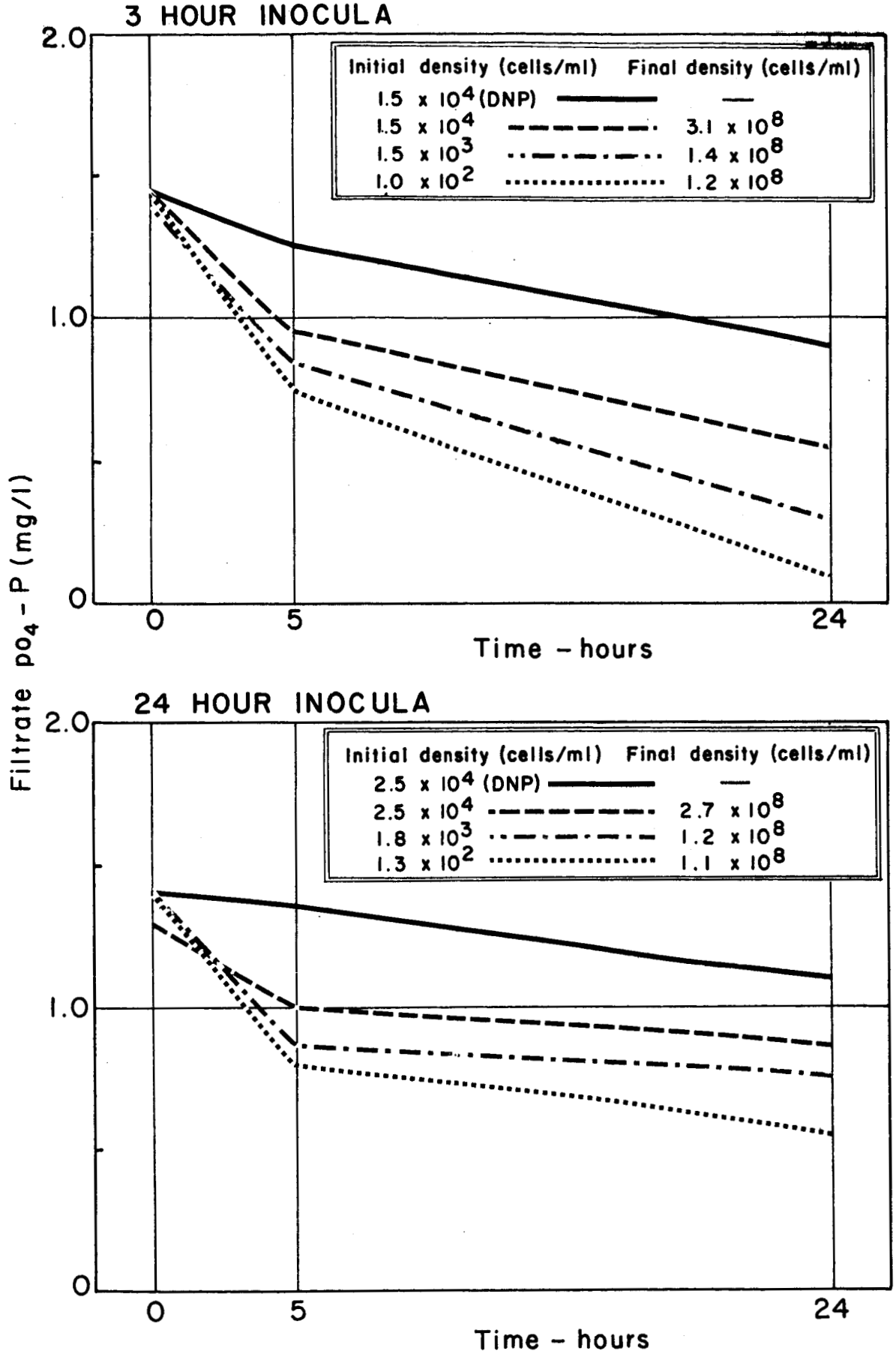

Figure No. 1 - Orthophosphate uptake by aerating (92 ml/min) cultures of E. Coli seeded from 3 hour and 24 hour cultures grown in low-phosphate M9.

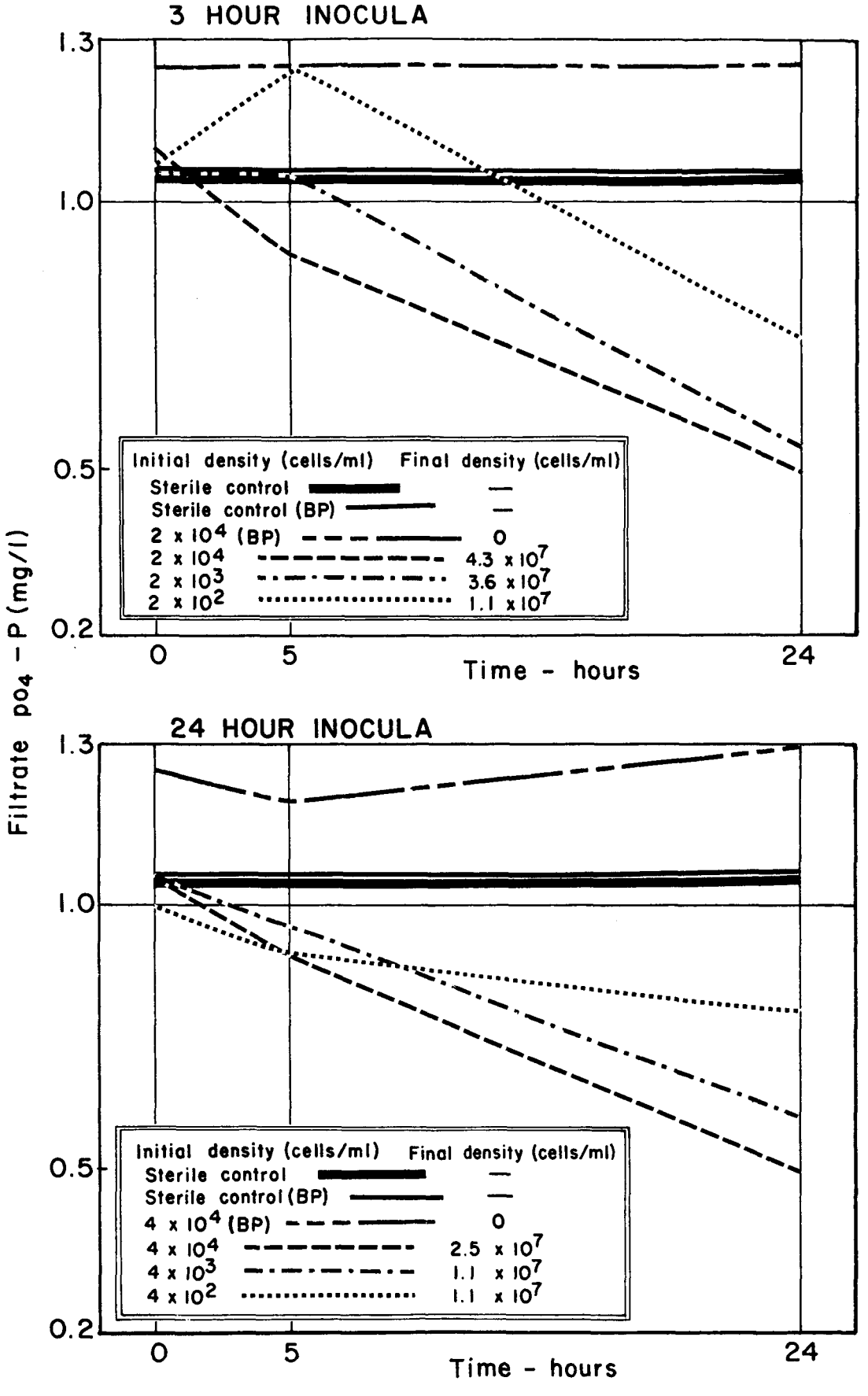

Figure No. 2 – Orthophosphate uptake by non-aerating cultures of E. Coli seeded from 3 hour and 24 hour culture grown in low-phosphate M9.

The age of the inoculum did not influence phosphate uptake as it did in the case of the aerating cultures. Contrary to the observations on aerated cultures, uptake varied directly with size of inoculum.

The inoculated controls poisoned with BP showed no uptake of phosphate. Instead, their dissolved phosphate levels were consistently higher than those of the sterile controls, demonstrating that phosphate leaked out from the poisoned cells almost immediately. This, even more forcefully than the DNP, showed the biological nature of the uptake.

2. Phosphate Uptake by Clostridium tetanomorphum

C. tetanomorphum was subcultured from cooked meat medium into low-phosphate (1.0 mg. PO_4-P per liter) M9 containing 0.2% glucose and 0.01% Na-thioglycollate (MTG-9) under anaerobic conditions obtained by flushing with N_2 gas 10 minutes before and after inoculation. The gassing lines were closed afterward to prevent diffusion of oxygen into the culture medium. A 24-hour culture containing approximately 5×10^7 cells per ml. was washed and serially diluted with MTG-9. One-tenth ml. aliquots of the diluted culture were added to 40 ml. MTG-9 medium to obtain approximate cell concentrations of 1×10^4, 1×10^3 and 1×10^2 per ml., and anaerobiosis was reestablished. Since this anaerobic organism is slower growing than E. coli, the 24-hour culture was used as the "young inoculum." The parent culture served as the "old inoculum" after 48 hours. The approximate density of the 48-hour inoculum was 9×10^8 cells per ml. Zero time cell numbers in 40 ml. MTG-9 were

1×10^4, 1×10^3 and 1×10^2 per ml. for both inocula. The results are shown in Figure No. 3.

Phosphate uptake was noticed at five-hour incubation from cultures of 24-hour inoculum. The corresponding cultures from 48-hour inoculum did not show any phosphate uptake. No final cell counts were made and it is possible that the cells died. The fact that phosphate leakage varied directly with size of inoculum indicates this was the case. The pattern of phosphate uptake in the 24-hour inocula cultures followed the aerated E. coli culture, the greatest uptake being exerted by the culture derived from the smallest inoculum.

The mode of action of Bard-Parker poison on anaerobic C. tetanomorphum seems to be different from that operating on the facultative E. coli. No clear pattern of phosphate leakage was observed in the former species. This observation and the possible death of the 48-hour inocula cultures argue for a repeat of this experiment.

B. ENGINEERING

The components required to test the feasibility of converting C^{14} beta particles into photons to permit the use of a photomultiplier readout system have been obtained. A plan for performing the test has been made.

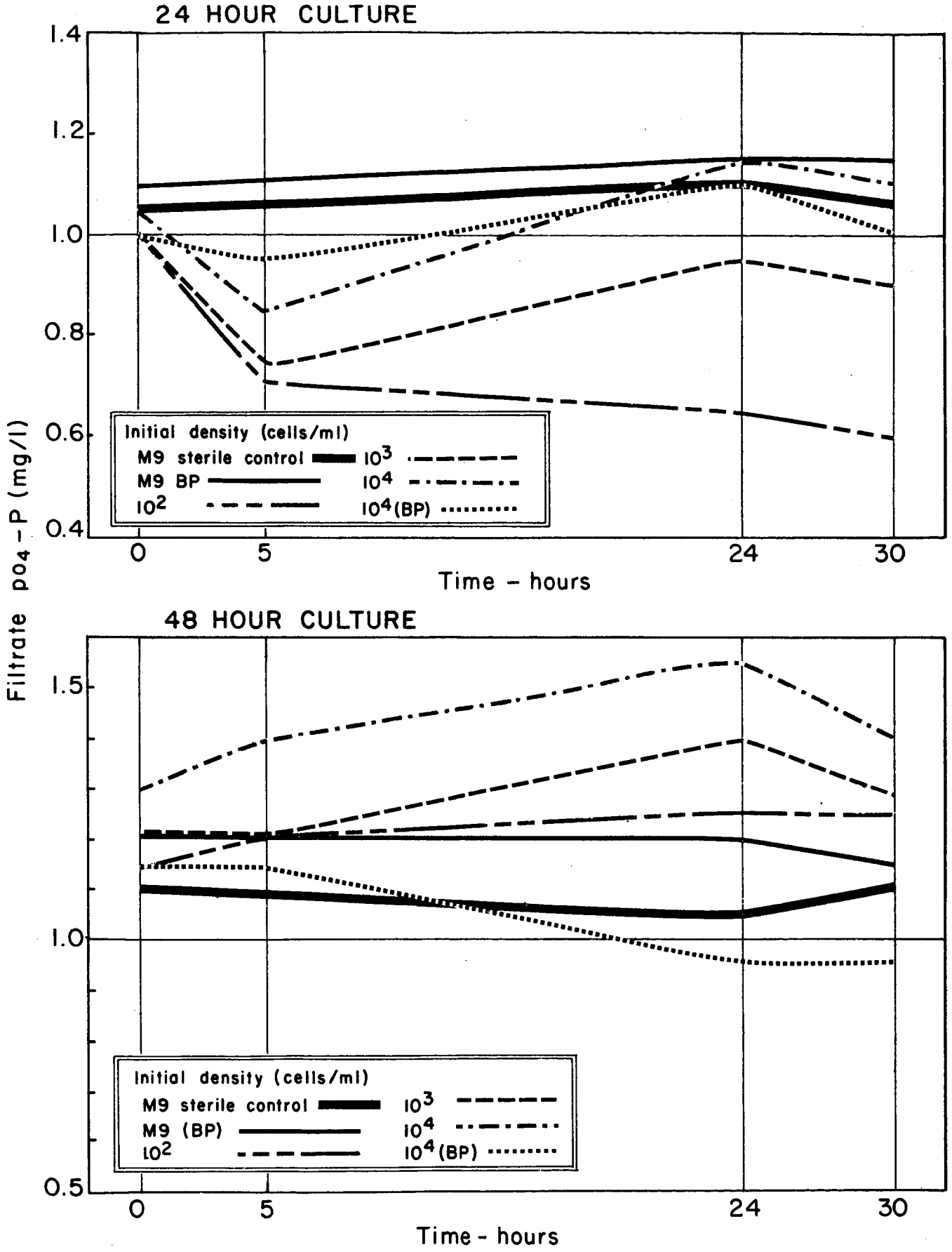

Figure No. 3 - Orthophosphate uptake by cultures of Cl. tetanomorphum seeded from 24 hour and 48 hour cultures grown in MTG - 9.

Respectfully submitted,

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HAZLETON LABORATORIES, INC.

PROJECT COST CONTROL

Supplement No.
Performance Period 10/1/66-5/30-67
Total Amount $97,000
Overhead Rate 119%
G & A Rate 14.5%
Fee Rate 7%

Client: National Aeronautics & Space Administration
Description: Development of an Automated Microbial Metabolism Laboratory
Contract Number: NASW-1507
Project Number: 604-112

* Other Direct Charges
1. Overtime
2. Professional Fees
3. Postage & Express
4.
5.

MONTHLY

Month	Yr.	Budget Total	Actual Billings Total	Direct Charges						Technical Overhead	G & A	Fee
				Labor	Materials and Supplies	Equipment	Travel	Other	*			
Oct. (5)	66	9,300	9,278	3,266	412		9			3,886	1,098	607
Nov.	66	11,692	3,247	1,133	165		5			1,348	384	212
Dec.	66	11,692										
Jan. (5)	67	14,615										
Feb.	67	11,692										
Mar.	67	11,692										

CUMULATIVE

Oct. (5)	66	9,300	9,278	3,266	412		9			3,886	1,098	607
Nov.	66	20,992	12,525	4,399	577		14			5,234	1,482	819
Dec.	66	32,684										
Jan. (5)	67	47,299										
Feb.	67	58,991										
Mar.	67	70,683										

Comments:

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